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PRINCIPAL INVESTIGATOR: Mark M. Rasenick, PhD

CONTRACTING ORGANIZATION:
University of Illinois

Chicago, IL 600612-7205

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14. ABSTRACT
The most significant findings during the period of this project were the discovery that lymphoblasts from autistic subjects could be used to determine overall neurotransmitter responsiveness. We had predicted that cells from autistic subjects would sort out, particularly in the arena of serotonin sensitivity, from those cells obtained from autistic subjects with normal serum serotonin. This was not the case, as the sequellae of events following serotonin binding to 5HT1, 5HT2 or 5HT4,7 receptors was identical in cells from the high and low serotonin autistic subjects. However, particularly for 5HT1 signaling, both autistic groups handily separated from the control. Thus, it appears that we have developed a cultured cell system that is both easy to manipulate and useful for studying serotonin signaling in autism.

15. SUBJECT TERMS
autism, lymphoblast, G protein, adenylyl cyclase, AMP, GPCR, phospholipase c

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INTRODUCTION

Distinct lines of neurochemical, behavioral, and now molecular genetic evidence implicate dysfunction of the serotonin system in autism spectrum disorders (ASD), and specifically in restricted and repetitive behaviors (RRBs). Discovery of elevated platelet serotonin (5HT) in ~25-30% of individuals with autism is one of the seminal findings in neuropsychiatric research. Autism is the most heritable complex neuropsychiatric disorder, and platelet 5HT levels are also extremely heritable (Abney, et.al. 2001). Further, elevated platelet 5HT is associated with familial recurrence risk, both in autism and in obsessive compulsive disorder (OCD). The relationship between autism and OCD is reflected in correlations between restricted and repetitive behaviors (RRBs) in probands with autism and OC symptoms in their parents (Abramson, et.al. 2005). Rare mutations in the serotonin transporter (SERT) gene (SLC6A4) have recently been identified that display a common pattern of elevated 5HT transporter activity and produce a phenotype of autism with RRBs or OCD (Sutcliffe, et.al. 2005).

An index of Insistence on Sameness (IS) from the ADI-R (Shao, et.al, 2003) and from the RBS-R (Lam, et.al. 2007) captures the symptoms that are often most distressing to patients and their families, however, deciphering the relationships between autism and a dysregulated 5HT system requires critical neurochemical phenotypes that will allow the underlying common mechanisms to be elucidated. For example, studies of platelet 5HT levels in inbred and outbred populations pointed to the integrin β3 gene (ITGB3) as a quantitative trait locus for platelet 5HT levels (Weiss, et.al, 2004). Further SERT and ITGB3 physically interact in the platelet, and that the absence of Itgb3 in mouse brain significantly diminishes SERT activity (Carneiro, et.al, 2008).

Other studies have found decreased 5HT2A receptor binding in the platelet that correlates between boys with autism and their fathers and parallels decreased receptor binding in recent brain studies. We suggest a dysregulated 5HT system, IS, and autism susceptibility are related. Furthermore, we hypothesize that variation within components of the 5HT signaling system is likely to contribute to autism susceptibility as well.

BODY Statement of Work

Weeks 1-16

Grow 10 lines each of normal and high-serotonin lymphoblasts from autistic subjects. Compare agonist (5HT4 and 5HT 1 stimulation and inhibition of adenylyl cyclase and 5HT2 stimulation of PLCb1 from the two populations. Also compare receptor-activated GTPgS binding between the two groups.

Weeks 16-40

Complete the rest of the cell lines from each group making necessary modification as learned from the initial studies. Treat cells with escitalopram or r-citalopram and test for increased coupling between G proteins and effector molecules

Weeks 40-52

Select 5 lines from each group and transfect with GFP-Gsa. Determine lipid raft distribution and changes in raft localization in response to agonist

Data are described below. We were not able to do more than 5 lines of each cell type, as the growth conditions were, initially, challenging. This was worked out and the cells are growing well and giving us good data (see below).

KEY RESEARCH ACCOMPLISHMENTS

Preliminary Data

Over 200 children and adolescents have been assessed with the research diagnostic tools, the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) at UIC as part of the UIC Autism Center of Excellence (UIC-ACE). Blood was sent to the NIMH genetics repository at Rutgers, where lymphoblast cell lines were established. We have grown cells from high 5HT/high IS and normal 5HT/moderate IS subjects and analyzed them as described below.

Our emphasis has been on the role of membrane microdomains (lipid rafts) in modulating G protein signaling (see Allen et.al, 2007), and a recent focus has been on 5HT4,6 and 7 receptors, which signal through Gsα. Gsα coupling to adenylyl cyclase is augmented when released from lipid rafts (Allen et.al, 2009). Further, Gsα is more highly ensconced in lipid rafts in certain psychiatric diseases, such as depression, and chronic treatment with antidepressants, including SSRIs, fosters translocation of Gsα to non-raft membrane domains (Zhang and Rasenick, 2010).

Future considerations

Lymphoblasts are not brains, and the translation to brains requires some faith on the part of both investigator and reviewer. Nonetheless, lymphocytes and platelets from patients with a number of psychiatric diseases have been used to search for biomarkers and establish altered receptor displays or signaling pathways. While viral transformation may change the cells, G-coupled protein receptor (GPCR) expression in lymphocytes and lymphoblasts are similar.

In order to address this concern, selected characterized cell lines will be reprogrammed, initially into pluripotent stem cells and then into neurons. This is no trivial exercise and there is some question that lymphoblasts can be infected with the “reprogramming” viral cassette (available from Sigma). We have been assured by Rudolph Jaenisch (personal communication) that this is indeed feasible (Staerk et.al., 2010) and Rusty Gage has told us that he will be glad to help us transform the induced pluripotent stem cells into neurons. We are aware that the efficiency of such an induction is very small, but if, indeed, the cell lines are useable, then the cell numbers are considerable. If not, we do have the ability to draw blood and collect lymphocytes from many of the subjects from whom we have lymphoblasts available.

By comparing lymphoblasts from the High- and Low-IS and high and low serotonin subjects, we hope to develop a clearer understanding of serotonin signaling in autism. Since many of the current drugs for autism influence the serotonin signaling system, it is thought that such an improved understanding will help us to develop more individualized therapy for these children and develop improved medications.

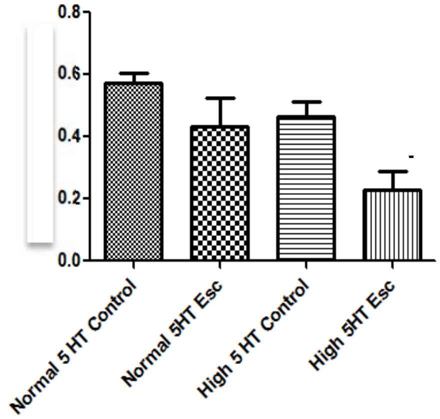

Figure 1 (above). Escitalopram translocates Gsα in cells obtained from High 5HT subjects. Lymphoblasts from High 5HT/High IS or Normal 5HT/moderate IS (two lines each) were grown in suspension and treated with escitalopram or control (r-citalopram, @10 μM) for 3days, harvested and membranes were prepared. Lipid raft fractions were prepared by sucrose density sedimentation and the extent of Gsα ensconsed in rafts (determined with quantitative immunoblots) is described in the figure. Escitalopram was effective in only in the high 5HT group. * $p < 0.01$; High 5HT control vs Normal control $p < 0.05$

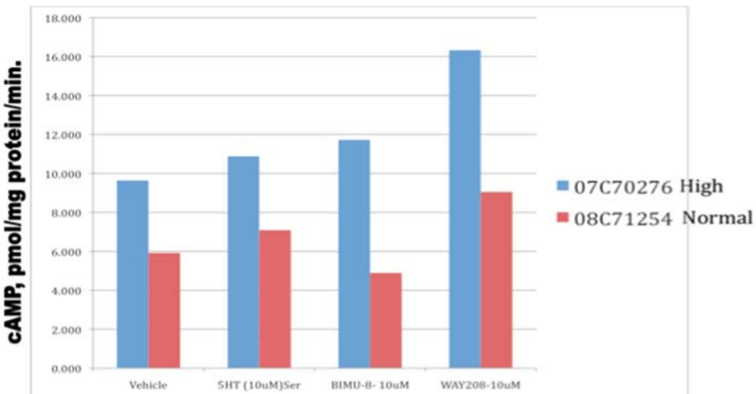

Figure 2 (above). Elevated response to 5HT4 and 5HT agonists in lymphoblasts from a high 5HT/high IS subject. Lymphoblasts from a high and normal 5HT subject were grown in suspension for 4 days, harvested and membranes were prepared and assayed for adenylyl cyclase in the presence of the indicated agonist (ser, 5HT; BIMU-B, 5HT4; WAY 408, 5HT6). Consistent with the results in figure 1, heightened responsiveness to Gs-coupled 5HT-activated adenylyl cyclase is seen in cells where Gsα is displaced from lipid rafts (where it coulples more effectively with adenylyl cyclase). Assays were performed twice, in triplicate.

REPORTABLE OUTCOMES

Two abstracts have been presented concerning this work. One was at the Brain Research Foundation symposium and the other was at the Molecular Pharmacology Gordon Conference. Natalie Cook was the presenting author on each. She received a Carl Storm Minority Fellowship to present this work at the Gordon Conference.

Drs. Cook and Rasenick submitted a Letter of Intent to the Simons foundation for funds to continue this work. The letter of intent was accepted and they were asked to submit a full proposal. A decision is still pending.

CONCLUSION

Lymphoblasts had been collected from patients only to generate genetic material. We have demonstrated, for the first time, that lymphoblasts prepared from patient material, can be used as a cell biological model for disease, which will be useful for both understanding disease process and for providing model systems to test novel therapeutic mechanisms.

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LIST OF PERSONNEL RECEIVING PAY FROM GRANT

Edwin Cook, MD
Mark M. Rasenick, PhD
Riti K. Chokshi
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Amanda M. Lathe